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Research Note

Scanning Electron Microscopy Study of a Copulating Monorchiid (Trematoda: Digenea)

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ABSTRACT: During a survey of parasites of fishes from the Kuwaiti coast of the Arabian Gulf, a pair of digeneans were found in copulation in the intestine of 1 of 4 *Gnathodon speciosus*; the digeneans were recovered and studied by scanning electron microscopy (SEM). SEM micrographs suggest a 1-way transfer of sperm and reveal the presence of longitudinal ridges with openings or depressions in the metratermal segment of the terminal organ of the recipient. The digeneans were identified as monorchiid species of *Lasiotocus*. This is the first evidence of copulation in a digenean demonstrated by SEM.

KEY WORDS: *Lasiotocus* sp., Monorchiidae, scanning electron microscopy, copulation, metraterm, *Gnathodon speciosus*, marine fish.

Trematodes, with few exceptions, are hermaphroditic flatworms that are capable of both cross- and self-fertilization. Most investigators believe that cross-fertilization is the rule. In cross-fertilization, sperms are transferred by the process of copulation from 1 individual to another in 1 direction or by reciprocal exchange.

The act of copulation has rarely been observed directly. Among the earlier studies are those of Fuhrmann (1930) on *Prosotocus confusus* (Looss, 1894) and Rausch (1947) on *Microphallus opacus* (Ward, 1894). Palombi (1932) reported copulation via Laurer's canal in *Diphtherostomum brusinae* (Stossich, 1889) and *Haploporus benedeni* (Stossich, 1887) and through the uterus in *Podocotyle fractum* (Rudolphi, 1819). (For more recent reviews dealing with copulation and fertilization, see Fried and Harris, 1971; Nollen 1983, 1997.) It is very probable, however, that most acts of copulation occur through the genital atrium by insertion of the cirrus or ejaculatory duct into the metraterm or the uterus. Here, we present an observation, which we consider direct evidence, of copulation through the metraterm in a monorchiid.

During the course of a survey of helminth parasites of Kuwaiti marine fishes conducted between October 1992 and December 1994, 4 golden trevally, *Gnathodon speciosus* (Forsskål, 1775) (Carangidae), were found to harbor monorchiids; in 1 of these hosts, a pair of digeneans in copula and 8 unpaired specimens were found. The digeneans were washed in saline, fixed in alcohol–formaldehyde–acetic acid, and stored in 70% ethanol. The copulating pair was dried for scanning electron microscopic (SEM) examination using the critical point technique, coated with gold-palladium, observed, and photographed using a JEOL, JSM-6300 SEM. The other specimens, which seem to represent 2 species, were stained in alum carmine, dehydrated through an ascending series of ethanol, cleared in clove oil, and mounted in Canada balsam.

Scanning electron micrographs show the copulating pair in a parallel but reverse position, the anterior end of 1 facing the posterior end of the other (Fig. 1A, B). Both the large cirrus sac and the metraterm part of the terminal organs are extruded (Figs. 1, 2), with the cirrus sac hooking up with the metraterm in an apparent 1-way transfer. The 1-way transfer is in contrast with the 2-way transfer described by Fuhrmann (1930) in *Prosotocus confusus*. The micrographs (Figs. 1, 2) show a conspicuous preacetabular genital pore that is wide open. The impression is that the recipient (Fig. 1B) is responding to the approach of the cirrus of the partner (the insertor) by opening its genital pore and protruding the metraterm. In the fixed specimens, the genital pore is barely visible, and no evidence of any metratermal protrusion is seen. The micrographs (Figs. 3, 4) also reveal that the metraterm has longitudinal ridges with pores or depressions. The function of these ridges is not

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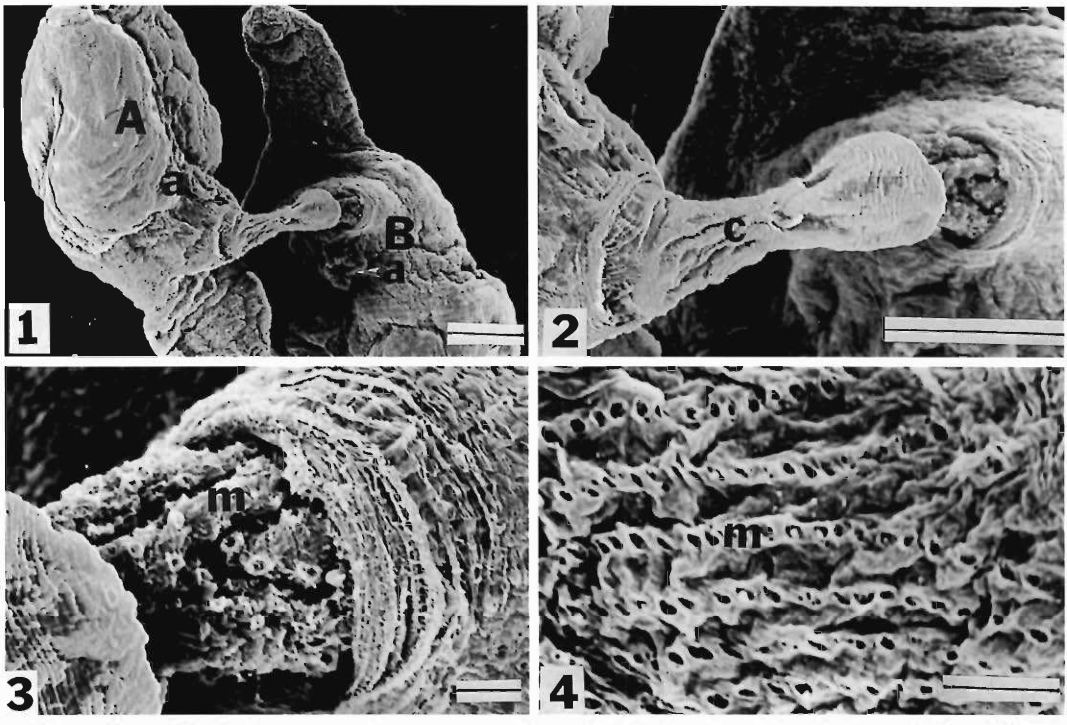

Figure 1–4. Copulating worms in reverse position, with the insertor (A) and the recipient (B). The anterior end of the insertor is pointing posteriorly, and the oral sucker of the recipient is near the upper edge of the picture; the acetabulum (a) is just posterior to the genital pore in B and the protruded cirrus in A. Scale bar = 100 μm. 2. Protruded cirrus (c) and its connection with the genital pore. Scale bar = 100 μm. 3. Protruded metraterm (m) showing ridges and pores or depressions. Scale bar = 10 μm. 4. Same worm as in Figure 3, from a slightly different angle, showing metratermal ridges, with pores or depressions, in a parallel arrangement. Scale bar = 1 μm.

known. Whether the pores or the depressions are openings of secretory glands or for chemoattractants can only be postulated. The production and secretion of chemoattractants has been reported previously (see Nollen, 1997).

The copulating digeneans were identified as a species of *Lasiotocus,* a monorchiid genus represented by at least 46 nominal species. The 10 specimens, including the copulating pair, recovered from 1 of 4 golden trevallys belong to 2 species. One species, represented by 1 specimen, is characterized by having small eggs, a small cirrus sac, and vitelline follicles that are confluent in the acetabular region; the other 7 specimens have larger eggs, a large cirrus sac, and vitelline follicles extending laterally from the acetabular level to the ovariotesticular level. Both species are to be described elsewhere. The assignment of the copulating pair to 1 or the other of these 2 species cannot be determined with certainty; in all likelihood, the specimen with the protruded cirrus, the insertor, (Fig. 1A) belongs to the species with the larger eggs and larger cirrus; whether the other partner belongs to the same species or to the other species cannot be determined from the micrographs. Both species were also found in the other 3 golden trevallys.

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Research Note

Seasonal Occurrence of Helminths of the Whistling Frog, *Eleutherodactylus johnstonei* (Amphibia: Leptodactylidae), in Bermuda

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ABSTRACT: Four hundred twenty-seven *Eleutherodactylus johnstonei* from 13 study sites in Bermuda were examined for helminths during March, July, and November 1995. Four nematode species, *Parapharyngodon garciae, Aplectana* sp., *Abbreviata* sp., and *Batracholandros* sp., and 2 trematode species, *Mesocoelium monas* and an unidentified species belonging to the family Opecoelidae, were found. The *Batracholandros* sp. and the opecoelid trematodes represent new records of parasitism in this frog species and for Bermuda.

KEY WORDS: *Eleutherodactylus johnstonei,* Leptodactylidae, Nematoda, *Parapharyngodon garciae, Aplectana* sp., *Abbreviata* sp., Oxyuridae, *Batracholandros* sp., *Mesocoelium monas,* Opecoelidae.

The leptodactylid frog *Eleutherodactylus johnstonei* Barbour, 1914, inhabits a number of Caribbean islands, including Anguilla, Antigua, Barbados, Barbuda, Grenada, Guadeloupe, Jamaica, Montserrat, Nevis, Saba, St. Barthelemy, St. Christopher, St. Eustatius, St. Lucia, St. Martin, and St. Vincent (Schwartz and Henderson, 1991). Between 1880 and 1886, *E. johnstonei* was introduced into Bermuda at Admiralty House, Pembroke Parish, probably from the Lesser Antilles (Wingate, 1965). The species is currently found throughout the 7 major islands comprising Bermuda.

Fifty-three *E. johnstonei* (16 males, 37 females) were collected from 2 sites during 20–24 March 1995: 12 (4 males, 8 females) were taken on the grounds of the Bermuda Biological Station for Research (BBSR) in St. George's Parish, and 41 (12 males, 29 females) were taken from a Harrington Sound banana patch in Smith's Parish. Mean (±SE) snout–vent length (SVL) = 22.5 ± 0.32 mm, range = 17–30 mm. Mean weight = 0.74 ± 0.03 g, range = 0.4–1.4 g. One hundred fifty-five frogs (99 males, 56 females) were taken during 10–21 July 1995 from 11 sites: BBSR (18), Harrington Sound banana patch (14), the Bermuda Perfumery in Hamilton Parish (18), Paget Cemetery in Paget Parish (20), Turks Head Lane in Devonshire Parish (20), Soundview Drive in Sandy's Parish (21), Fort Albert in St. George's Parish (10), St. David's Island in St. George's Parish (19), Lukes Pond Road in Southampton Parish (10), and Sea Swept Farm in Southampton Parish (5). Mean (±SE) SVL = 22.8 ± 0.24 mm, range = 16.5–33 mm. Mean (±SE) weight = 0.8 ± 0.03 g, range = 0.28–1.98 g. Two hundred nineteen frogs (88 males, 131 females) were taken during 18–24 November 1995 from 13 sites: Bermuda Perfumery (20), Soundview Drive (19), Lukes Pond Road (20), Sea Swept Farm (17), Cameron

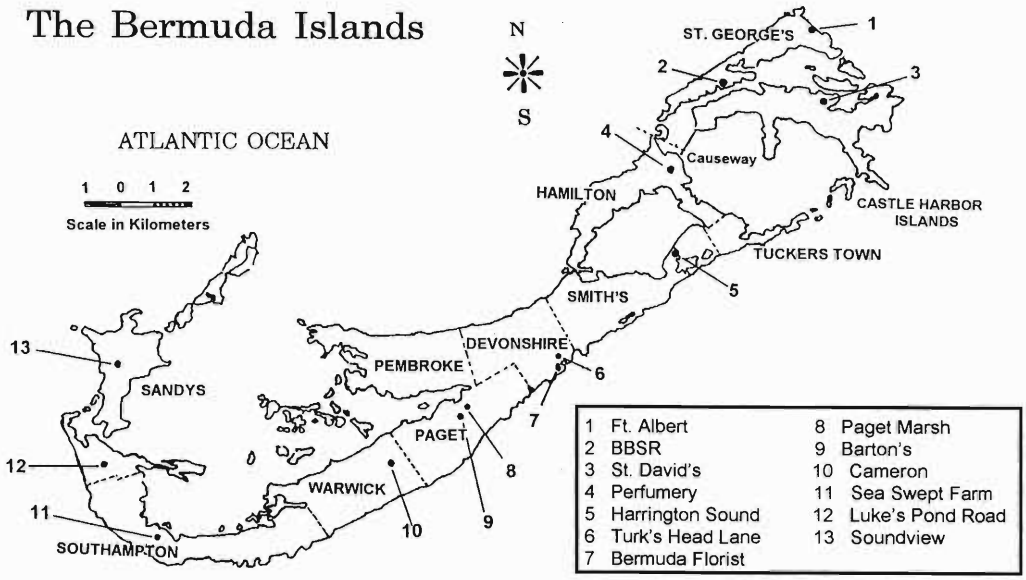

Figure 1. Study sites for *Eleutherodactylus johnstonei* in Bermuda.

in Warwick Parish (20), David Barton's in Paget Parish (16), Paget Marsh (10), Bermuda Florist in Devonshire Parish (20), Harrington Sound banana patch (20), St. David's Island (17), BBSR (20), and Fort Albert (20). Mean (±SE) SVL = 23.5 ± 0.22 mm, range = 15–34 mm. Mean (±SE) weight = 0.81 ± 0.03 g, range = 0.23–2.3 g.

Study sites are shown in Figure 1. Approximate straight-line distances from the BBSR are Ft. Albert, 2.5 km NE; St. David's, 2.5 km E; Bermuda Perfumery, 2.75 km SW; Harrington Sound banana patch, 4.5 km SW; Turks Head Lane and Bermuda Florist, 8.5 km SW; Paget Marsh and D. Barton's, 10.5 km SW; Cameron, 13.0 km SW; Sea Swept Farm, 18.5 km SW; Lukes Pond Road, 20.0 km SW; and Soundview Drive, 17.0 km SW.

All specimens were frozen immediately upon return to the BBSR. Later, each individual was thawed, measured, and weighed. The lungs, liver, gall bladder and bile duct, stomach, small intestine, large intestine, and urinary bladder were removed and examined separately under dissecting and compound microscopes. Nematodes were identified utilizing the standard glycerol wet-mount procedure. Trematodes were stained with Ehrlich's hematoxylin and mounted in Canada balsam. Terminology follows that of Bush et al. (1997). Selected helminths were deposited in the U.S. National Parasite Collection (USDA, Beltsville, Maryland): *Parapharyngodon garciae,* 87200; *Aplectana* sp., 87197; *Abbreviata* sp., 87196; *Mesocoelium monas,* 87204; and Opecoelidae, 87202, 87203.

Seasonal prevalence, mean abundance, and mean intensity for each helminth are given in Tables 1–3. For March, 49 of 53 (92%) *E. johnstonei* harbored helminths; for July, 130 of 155 (84%); and for November, 194 of 219 (89%).

Aplectana sp. (females only) was found in the small and large intestines in March: 17 of 53 frogs (32%), 4/16 (25%) males, 13/37 (35%) females; July: 54 of 155 frogs (35%), 38/99 (38%) males, 16/56 (29%) females; and November: 65 of 219 frogs (30%), 30/89 (34%) males, 35/131 (27%) females. Differences in seasonal infection rates (March, July, and November) were highly significant at the BBSR ($\chi^2 = 8.85$, 2 df, $P < 0.01$) but not at Harrington Sound ($\chi^2 = 1.04$). At the BBSR, differences in prevalence between March and November were highly significant (χ^2

Table 1. Seasonal prevalence, mean abundance, and mean intensity of *Aplectana* sp. infection in *Eleutherodactylus johnstonei* in Bermuda, 1995.

	March			July			November		
Study site	Prevalence (%)*	Mean Abundance ($\bar{x}$ ± SD)	Mean intensity (range)	Prevalence (%)*	Mean Abundance ($\bar{x}$ ± SD)	Mean intensity (range)	Prevalence (%)*	Mean Abundance ($\bar{x}$ ± SD)	Mean intensity (range)
Ft. Albert				60 (6/10)	4.1 ± 7.2	6.8 (1–24)	85 (17/20)	4.9 ± 4.7	5.8 (1–20)
BBSR	50 (6/12)	3.4 ± 6.9	6.8 (1–25)	22 (4/18)	0.3 ± 0.7	1.5 (1–2)	5 (1/20)	0.05 ± 0.2	1.0
St. David's				63 (12/19)	3.8 ± 5.3	6.0 (1–21)	41 (7/17)	1.6 ± 2.5	3.9 (1–8)
Perfumery				17 (3/18)	0.2 ± 0.5	1.3 (1–2)	25 (5/20)	0.6 ± 1.1	2.2 (1–4)
Harrington Sound	27 (11/41)	3.7 ± 1.3	2.0 (1–7)	36 (5/14)	0.6 ± 1.0	1.8 (1–3)	20 (4/20)	0.5 ± 1.4	2.5 (1–6)
Turks Head				50 (10/20)	0.9 ± 1.2	2.0 (1–4)			
Bermuda Florist							10 (2/20)	0.2 ± 0.5	1.5 (1–2)
Paget Marsh				20 (4/20)	0.5 ± 1.3	2.2 (1–6)	40 (4/10)	3.0 ± 1.7	3.0 (1–4)
Barton's							19 (3/16)	1.3 ± 0.6	1.3 (1–2)
Cameron							10 (2/20)	0.4 ± 1.4	4.0 (2–6)
Sea Swept				20 (1/5)	0.6 ± 1.2	3.0	12 (2/17)	0.4 ± 1.4	3.5 (1–6)
Lukes Pond				10 (1/10)	0.1 ± 0.3	1.0	5 (1/20)	0.05 ± 0.2	1.0
Soundview				29 (8/21)	0.8 ± 1.3	2.0 (1–5)	100 (19/19)	4.3 ± 3.3	4.3 (1–12)

* Numbers in parentheses are no. frogs infected/no. frogs examined.

Table 2. Seasonal prevalence, mean abundance, and mean intensity of *Abbreviata* sp. infection in *Eleutherodactylus johnstonei* in Bermuda, 1995.

	March			July			November		
Study site	Prevalence (%)*	Mean Abundance ($\bar{x}$ ± SD)	Mean intensity (range)	Prevalence (%)*	Mean Abundance ($\bar{x}$ ± SD)	Mean intensity (range)	Prevalence (%)*	Mean Abundance ($\bar{x}$ ± SD)	Mean intensity (range)
Ft. Albert				60 (6/10)	1.4 ± 1.9	2.3 (1–6)	45 (9/20)	1.8 ± 3.4	3.9 (1–12)
BBSR	17 (2/12)	10.4 ± 23.7	62.5 (50–75)	56 (10/18)	2.9 ± 4.8	5.3 (1–16)	95 (19/20)	11.6 ± 13.4	11.6 (2–53)
St. David's				74 (14/19)	2.3 ± 2.6	3.1 (1–9)	47 (8/17)	2.4 ± 5.2	5.1 (1–22)
Perfumery				83 (15/18)	8.2 ± 8.8	9.8 (1–38)	75 (15/20)	9.5 ± 11.1	12.6 (1–49)
Harrington Sound	76 (31/41)	6.9 ± 8.4	9.2 (1–30)	79 (11/14)	5.4 ± 5.4	6.9 (1–16)	95 (19/20)	8.6 ± 6.4	9.1 (1–22)
Turks Head				80 (16/20)	4.5 ± 5.7	18.0 (1–21)			
Bermuda Florist							70 (14/20)	5.8 ± 7.5	8.3 (2–30)
Paget Marsh				65 (13/20)	3.5 ± 5.0	5.3 (1–18)	10 (1/10)	0.1 ± 0.3	1.0
Barton's							94 (15/16)	27.7 ± 18.0	29.5 (1–65)
Cameron							90 (18/20)	5.6 ± 5.6	6.2 (1–21)
Sea Swept				100 (5/5)	9.6 ± 10.1	9.6 (2–28)	65 (11/17)	2.2 ± 2.6	3.4 (1–8)
Lukes Pond				70 (7/10)	2.4 ± 3.6	3.4 (1–12)	95 (19/20)	8.1 ± 6.8	8.5 (2–26)
Soundview				52 (11/21)	2.9 ± 3.7	5.5 (1–11)	79 (15/19)	4.1 ± 7.8	5.2 (1–36)

* Numbers in parentheses are no. frogs infected/no. frogs examined.

Table 3. Seasonal prevalence, mean abundance, and mean intensity of *Parapharyngodon garciae* infection in *Eleutherodactylus johnstonei* in Bermuda, 1995.

	March			July			November		
Study site	Prevalence (%)*	Mean Abundance ($\bar{x}$ ± SD)	Mean intensity (range)	Prevalence (%)*	Mean Abundance ($\bar{x}$ ± SD)	Mean intensity (range)	Prevalence (%)*	Mean Abundance ($\bar{x}$ ± SD)	Mean intensity (range)
Ft. Albert				0 (0/10)			0 (0/10)		
BBSR	42 (5/12)	0.5 ± 0.7	1.2 (1–2)	33 (6/18)	0.6 ± 0.9	1.7 (1–3)	50 (10/20)	2.7 ± 1.6	2.7 (1–5)
St. David's				42 (8/19)	0.6 ± 0.8	1.4 (1–2)	24 (4/17)	0.3 ± 0.6	1.3 (1–2)
Perfumery				33 (6/18)	0.4 ± 0.6	1.2 (1–2)	20 (4/20)	0.2 ± 0.4	1.0 (1–1)
Harrington Sound	42 (17/41)	0.6 ± 0.9	1.5 (1–4)	14 (2/14)	0.1 ± 0.4	1.0 (1–1)	45 (9/20)	0.8 ± 1.0	1.7 (1–4)
Turks Head				20 (4/20)	0.3 ± 0.6	0.7 (1–2)			
Bermuda Florist							20 (4/20)	0.3 ± 0.5	1.3 (1–2)
Paget Marsh				5 (1/20)	0.1 ± 0.4	2.0	10 (1/10)	0.1 ± 0.3	1.0
Barton's							13 (2/16)	0.1 ± 0.3	2.0 (1–1)
Cameron							35 (7/20)	0.6 ± 0.9	1.7 (1–3)
Sea Swept				20 (1/5)	0.2 ± 0.4	1.0	59 (10/17)	1.1 ± 1.1	1.8 (1–3)
Lukes Pond				10 (1/10)	0.2 ± 0.6	2.0	55 (11/20)	0.9 ± 0.9	1.7 (1–2)
Soundview				0 (0/21)			0 (0/19)		

* Numbers in parentheses are no. frogs infected/no. frogs examined.